

# **ENVIRONMENTAL IMPACTS OF PETROLEUM PRODUCTION: THE FATE OF PETROLEUM AND OTHER ORGANICS ASSOCIATED WITH PRODUCED WATER FROM THE OSAGE-SKIATOOK PETROLEUM ENVIRONMENTAL RESEARCH SITES, OSAGE COUNTY, OKLAHOMA**

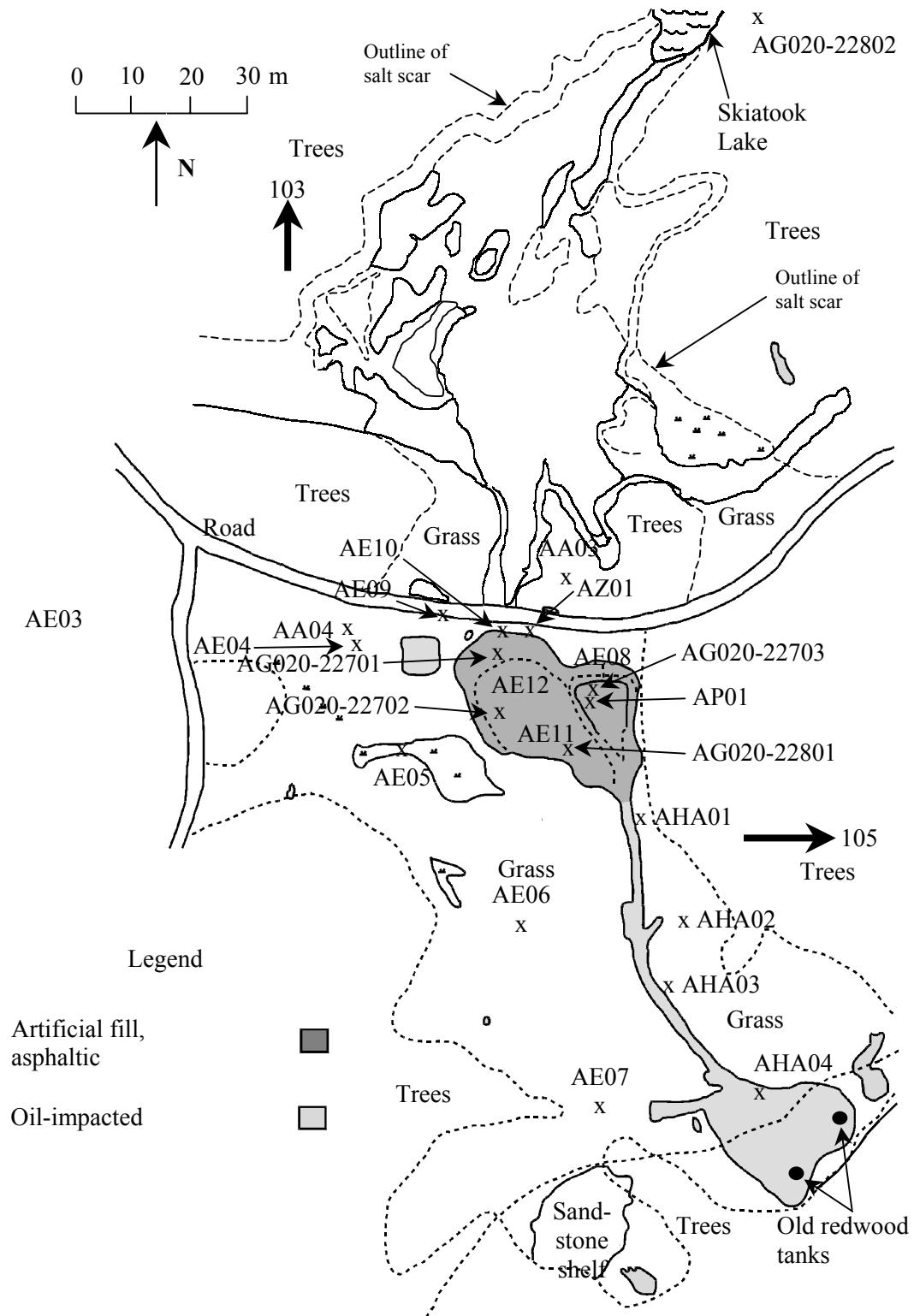
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## **ABSTRACT**

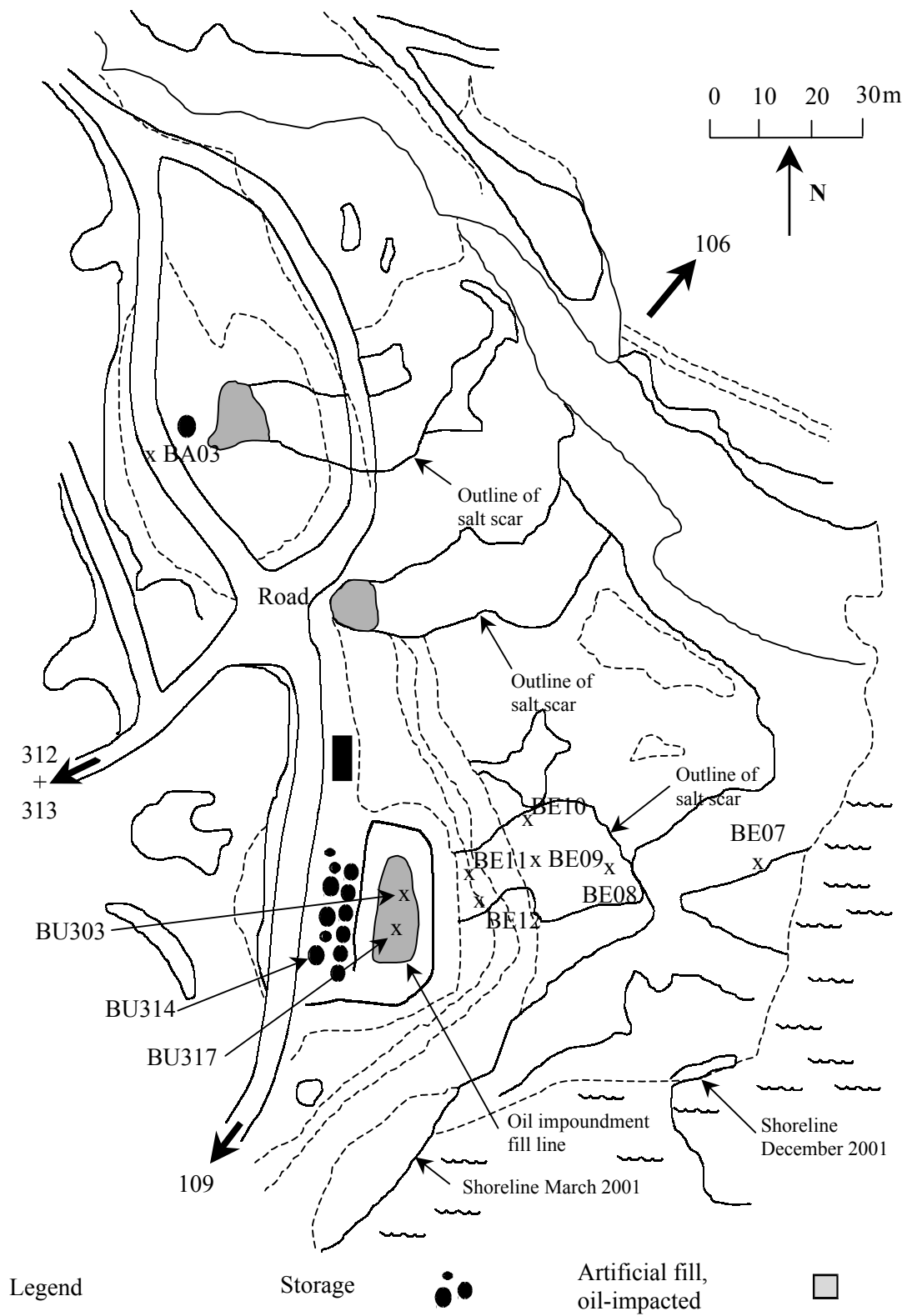
During a multidisciplinary study at two oil production sites of differing ages near Lake Skiatook, Osage County, Oklahoma, a series of oil, water, brine, and soil samples were characterized and analyzed for geochemical parameters that are indicative of microbial activity. Characterization of the resident microbial populations was completed for a number of these samples. The area source oils are a paraffinic-naphthenic light oil, containing *n*-alkanes as the dominant components unimpacted by biodegradation. The oils at the inactive site are similar although vary in stages of biodegradation. Some nearby oils at the active site do not correlate, suggesting that there is not significant subsurface transport of the spilled oils. Microbial populations at both sites are degrading the water-soluble crude oil compounds. The geochemical evidence and make-up of the microbial populations at both sites indicate that the systems are slightly different but both are thermodynamically poised at the level of iron reduction.

## **INTRODUCTION**

We are involved in a multidisciplinary investigation to study the transport, fate, and natural attenuation of inorganic salts, trace metals, radionuclides and organic compounds present in produced water and their impacts on surface and ground water and soils on the local ecosystem at the Osage-Skiatook Petroleum Environmental Research (OSPER) Site A (Figure 1) and Site B (Figure 2). About one hectare of land at each of the sites is affected by salt scarring, soil salinization, and petroleum contamination. The main environmental concern results from the fact that the sites are adjacent to Skiatook Lake, a 4,250-hectare reservoir, that provides drinking water to the local communities and is a major recreational fishery. Petroleum wells and tank batteries at Site A have been inactive for some time and the bulk of the hydrocarbon (now degraded and weathered oil) and produced water releases occurred more than 60 years ago. One pit at this site however, contains relatively fresh asphaltic oil and high salinity brine. Site B includes an active production tank battery and adjacent brine and oil pit, an inactive tank battery and an injection well with a small brine pit.



**Figure 1.** Sample locations for this portion of the study and important features at the Site A location. Large arrows point to sample locations that are off the map area.



**Figure 2.** Sample locations for this portion of the study and important features at the Site B location. Large arrows point to sample locations that are off the map area.

For this portion of the study, samples of sediments, water, oil, and brine were obtained from several oil wells at the Site B and areas adjoining the Sites A and B, from the main pit at the Site B, from the asphaltic pit and adjacent weathered oil pit at the Site A and from several of the 40 boreholes (1-71 m deep), recently drilled and completed. Samples of sediments and/or water from these selected boreholes and from the brine and oil pits were obtained for characterization of the microbial population. Water samples for dissolved organics were obtained from selected boreholes with high salinity water and measurable hydrocarbon gases in the unsaturated zone. Soil and rock core samples were obtained from these selected boreholes to determine the amount and composition of oil sorbed onto the sediments.

Results completed to date show the crude oil source throughout the area is a typical paraffinic-naphthenic light (API gravity of ~35) oil, containing *n*-alkanes as the dominant components. Even though petroleum production is from shallow sandstones (450-524 m Site A and 260-305 m Site B), the oils show no sign of biodegradation, indicating that microbes are not particularly active in the associated high salinity (~150,000 mg/L total dissolved solids) brine and also possible inhibition by the hydrocarbon oil phase (currently under investigation). Microbial action, volatilization and water washing are likely responsible for the transformation of source oil to the asphaltic and weathered oil observed on the surface at Site A. The leakage of oil with brine from the main active pit at Site B is indicated by the detection of a thin, but discrete oil phase in at least one borehole, the presence of hydrocarbon gases in several boreholes and the smell of oil in many sediment cores from the impacted area located downslope from this pit. The measured concentrations of dissolved organic carbon (DOC), acetate and other organic acid anions, benzene, toluene, ethylbenzene, xylenes (BTEX), phenols and other organics in the source brine are relatively low, but their values in water samples from the impacted areas are higher and indicative of microbial activity.

## **MATERIALS AND METHODS**

### **Microbial Number Determinations**

Microbial numbers in both water and sediment samples were determined using a five-tube most probable number (MPN) analysis. Samples were serially diluted by an order of magnitude into pre-reduced anaerobically sterilized (PRAS) mineral salts solution. Aliquots of the dilutions were inoculated into six different media, designed to promote growth and the enumeration of aerobic heterotrophic, facultative and strict anaerobic heterotrophic, denitrifying, facultative and strict iron-reducing, sulfate-reducing, and methanogenic microorganisms (1).

### **Core Sample Collection**

Sediment samples were collected in clear polycarbonate liners using a Geoprobe<sup>®</sup> or with a hand auger using similar polycarbonate liners. The liners were cut and, with O<sub>2</sub>-free N<sub>2</sub> flowing over the sediment, about 10 g of sediment was scooped out with a sterile spatula and placed in 25 x 142 mm anaerobic isolation roll streak tubes (Bellco Glass Inc., Vineland, N.J.) filled with 20 mL of prereduced, anaerobically sterilized, mineral salts solution (2) and 10 mg/L Tween 80<sup>®</sup> (a nonionic surfactant added to help remove microorganisms from the sediment) (1). The tubes were then sealed, and

allowed to stand for 2 hr to permit penetration of the Tween 80 into the sample. The tubes were opened with O<sub>2</sub>-free N<sub>2</sub> flowing over the surface and sonicated (10W for 30 s) to dislodge microorganisms into the mineral salts using a Branson Sonifer, Model 200, equipped with a microtip (Branson Corp., Danbury, Conn.). The samples were stored no more than 4 hr on ice (4°C) before microbial enumeration.

## **Water Sample Collection and Analysis**

Water samples were removed using a peristaltic pump equipped with Teflon-lined tubing that had been previously cleaned with a detergent solution, rinsed with tap water and then with deionized water. Water samples were generally collected after achieving stable pH and temperature values.

Water samples to be analyzed for low molecular weight organic acid anions were first collected and analyzed by ion chromatography (3). Inorganic cations were analyzed by inductively coupled plasma/mass spectrometry (4). Both cation and anion samples were chilled at 4°C until analyzed.

Water samples for dissolved gas analyses of methane (CH<sub>4</sub>) were collected in 60-mL syringes and injected into evacuated 30 mL serum bottles until atmospheric pressure was reached. Samples were preserved with 50 mg/L mercury using mercuric chloride. Dissolved CH<sub>4</sub> was analyzed by a gas chromatograph equipped with a thermal conductivity detector (5).

Twenty mL water samples for microbial enumeration were collected with disposable 60-mL sterile syringe and injected into a sterile 30-mL glass serum bottle. The serum bottles contained an oxygen (O<sub>2</sub>)-free nitrogen (N<sub>2</sub>) atmosphere sealed with a butyl rubber stopper and secured with an aluminum crimp seal. All water samples were stored on ice for no more than 4 hr before inoculating media onsite.

## **Production Oil Sample Collection**

Four oils in current production from local oil wells near the study area are included in the sample set. Samples from Site A and B included muds, rocks, sandy soil or sediment collected from surface accumulations or in cores, asphaltic surface oils, oil floating on water, and congealed oil from brine tanks. Table 1 describes the samples. Site samples were collected in pre-cleaned glass jars, refrigerated after collection, and then frozen until analysis. When the samples were unfrozen for analysis, any water was decanted off. One wet sediment sample (BE07) was air-dried and ground to about 30 mesh. Thirty grams was extracted with dichloromethane (DCM), filtered through glass wool. The filtrate used for analysis (below). All other site samples were extracted with DCM after thawing. If traces of water were present, the water was removed by addition of sodium sulfate, followed by filtration. Copper powder, pre-activated with concentrated hydrochloric acid, was also added to the DCM solutions before filtration in order to remove any possible traces of sulfur. The four production oils (25 mg each) were dissolved in DCM and treated with copper. Aliquots of each of the site samples were measured so that approximately 25 mg of extractable material was analyzed. For each sample its DCM solution was blown down to near-dryness under a gentle stream of pure nitrogen, and the residue dissolved in 5 mL of hexane.

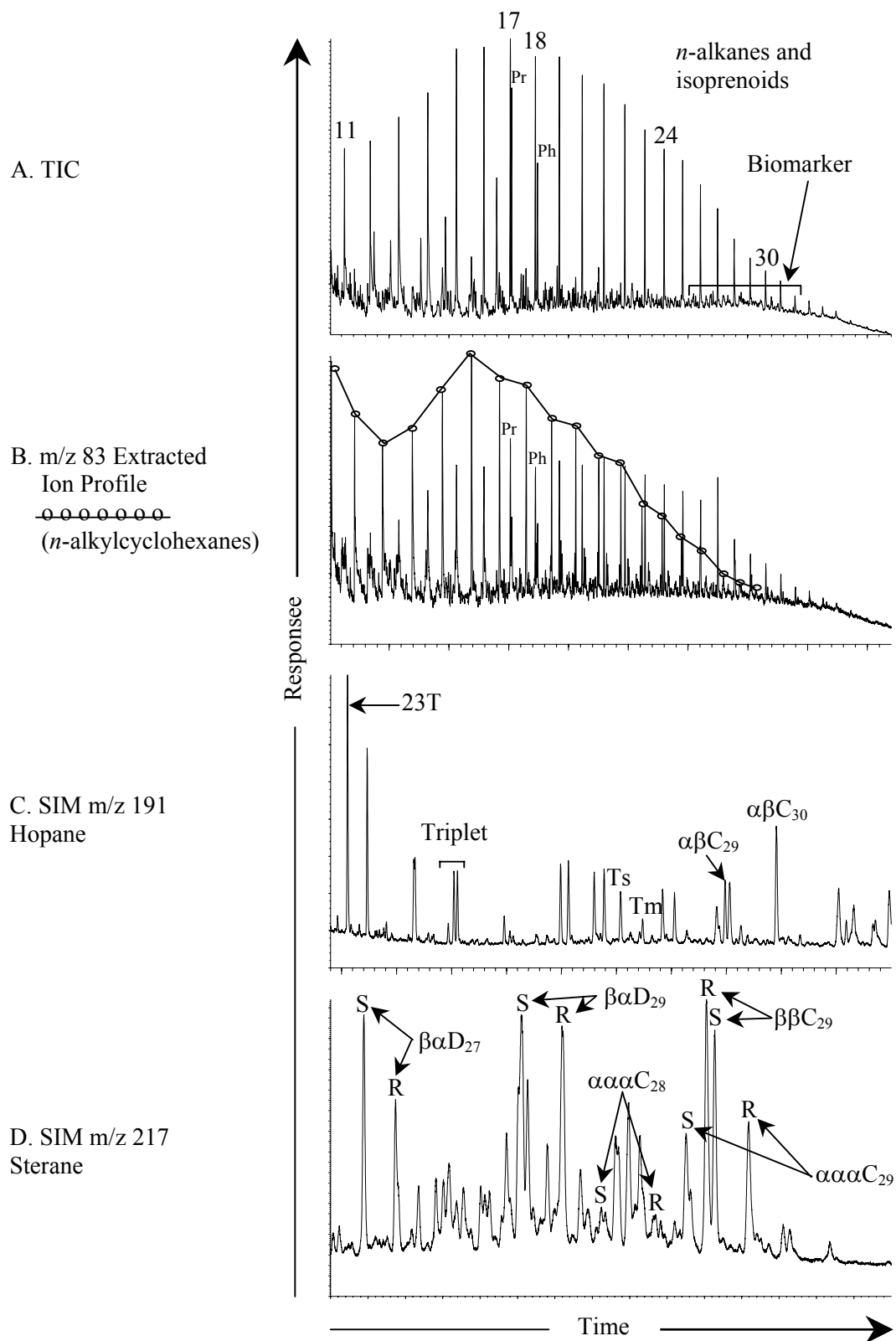
**Table 1.** Sample and site descriptions

Field designation	Description	Site location	Deg'n*
<u>Locally produced oils</u>			
103	Oil in current production	N of A-site	A
105	Oil in current production	E of A-site	A
106	Oil in current production	NE of B-site	A
109	Oil in current production	SW of B-site	A
<u>Site A (02OS-)</u>			
AG020-22701	Oil in mud, surface	Oil/fill, down slope, edge pit	B/A
AG01-22702	Oil in mud, surface	Oil/fill, down slope, old area	C+
AG020-22703	Oil in mud/sed., surface	Oil/fill down slope, new area	A/B
AG020-22801	Mature asphalt, solids	Oil/fill, down slope, old area	D+
AE12-436	Traces oil floating on water	Oil/fill, down slope, well	C+
AP01, Sludge Pit	Oil, sticky mud, 60 cm BLS**	Oil/fill, down slope, new area	A
AP01, Sludge Pit	Oil, sticky mud, 30 cm BLS	Oil/fill, down slope, new area	A
AZ01	Sandy sed./oil, 50 cm BLS	Oil/fill, down slope, edge pits	D&C+
AHA04-20 cm	Oil in mud, water, 20 cm" BLS	Oil-impacted soil, core, upslope	C++
AHA04-46 cm	Oil in sandy mud, 46 cm BLS	Oil-impacted soil, core, upslope	C+
AHA04-66 cm	Oil/ sed., water, 66 cm BLS	Oil-impacted soil, core, upslope	C/D+
AG020a-22802	Sand/sediment, 10 cm	Pit far down slope, near lake	D&A
AG020b-22802	Gray mud/clay, 40 cm	Pit far down slope, near lake	A
AE03	Old surface asphaltic tar	By well, W edge site A	D
<u>Site B (02OS-)</u>			
BE07	Wet soil, 1.2-2.1 m /66.5 cm	E of tanks and pit	C
BU-314	Residual oil	Brine tank battery (by pit)	A
BU-314	Congeaed oil with water	Brine tank battery (by pit)	A
BA03	Rocks/ oil, 5.7-5.8 m BLS	NW of brine tanks and pit	C
BU-303	Viscous surface tar	NW corner of site B	A
BU-317	Congeaed oil on water	Large main pit	A
312	Flocculent - water, 34.5 m	West of site B, deep well 2	C
313	Flocculent - water, 35.9 m	West of site B, deep well 2	C

\*Deg'n = Degradation State, defined in text

\*\*BLS = below land surface

The hexane solution was then loaded onto a liquid chromatography column layered with alumina and silica gel for compound class separation. The column was eluted with hexane and then 30% DCM in hexane, rinsing the 5 mL container with the 30% solution when the eluting solvent was changed. Two separate fractions were collected-saturate (hexane) and aromatic (30% DCM). These fractions were analyzed for aliphatic and aromatic hydrocarbons by gas chromatography/mass spectrometry (GC/MS) as Total Ion Chromatograms (TIC) and in the Selected Ion Monitoring mode (SIM). Compound identifications either were made by comparison with known standards or with published spectra. Chromatograms of a typical local production oil, including a TIC, an Extracted Ion Chromatogram (EIC) of  $m/z$  83 (alkylcyclohexanes), and a SIM chromatogram of terpanes ( $m/z$  191) and steranes ( $m/z$  217), with peaks of interest noted, are shown in Figure 3. The TICs were used to evaluate the level of weathering or degradation of the samples. The state of weathering (Table 1) ranged from A to D, where



**Figure 3.** Chromatograms of Production Oil 103. Most compounds of interest are identified in the text. Others:  $\beta\alpha D_{27}$  = 13 $\beta$ ,17 $\alpha$ (H)-diacholestane (S+R);  $\beta\alpha D_{29}$  = 24-ethyl-13 $\beta$ ,17 $\alpha$ (H)-diacholestane (S+R);  $\beta\beta C_{29}$  = 24-ethyl-14 $\beta$ ,17 $\beta$ (H)-cholestane (S+R).

A = no/low degradation, *n*-alkanes dominant; B = *n*-alkanes minor, isoprenoids becoming prominent; C = no *n*-alkanes, isoprenoids dominant; and D = highly degraded, almost no residual components except high-molecular-weight biomarkers. Some samples from the Site A included some anomalous high-end alkanes, indicated in Table 1 with a '+’.

Selected biomarker ratios, listed below, were calculated from the TIC and SIM chromatograms using peak heights. These biomarker parameters were used to correlate the samples, and included the following ratios (Table 2):

1. Pr/Ph, pristane/phytane. This ratio of two ubiquitous isoprenoid hydrocarbons is widely used as a source parameter (6).
2. 23T/C<sub>30</sub>, C<sub>23</sub>tricyclic terpane/17 $\alpha$ ,21 $\beta$ (H)-hopane. This ratio is a source parameter adapted from Peters and Moldowan (6).
3. Tm/Ts, 17 $\alpha$ -22,29,30-trisnorhopane/18 $\alpha$ -22,29,30-trisnorhopane. This ratio is used as both a source and maturity parameter (7).
4. Triplet, [C<sub>26</sub>-tricyclic terpane (S?) + C<sub>26</sub>-tricyclic terpane (R?)]/C<sub>24</sub>-tetracyclic terpane. This source parameter was used to distinguish coastal tar residues in Prince William Sound (8).
5. C<sub>30</sub>/C<sub>29</sub>, 17 $\alpha$ ,21 $\beta$ (H)-hopane/17 $\alpha$ ,21 $\beta$ (H)-30-norhopane. This ratio was used by Palacas et al. (9) as a source parameter.
6. C<sub>29</sub>S/(S+R), 24-ethyl-5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ (H)-cholestane (20S)/ 24-ethyl-5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ (H)-cholestane (20S+20R). This sterane epimer ratio is commonly used in petroleum geochemistry as a maturity parameter; the equilibrium value at full maturity is ~0.5 (10).
7. C<sub>28</sub>R/C<sub>29</sub>R, 24-methyl-5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ (H)-cholestane (20R)/ 24-ethyl-5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ (H)-cholestane (20R). This source parameter has been modified from discussions in Grantham and Wakefield (11) and Waples and Machihara (12).
8. RI, Refractory Index. This PAH ratio is a source parameter, the ratio of the major peak in the highly refractory C<sub>26</sub> to C<sub>28</sub> triaromatic sterane suite and in the monomethyl chrysenes (13).

## RESULTS AND DISCUSSION

### Microbial Populations

The general process of biodegradation of the water-soluble fraction (WSF) of crude oil occurs under specialized conditions. In the saturated subsurface, WSF compounds are used by the microorganisms as an electron donor for energy, and dissolved O<sub>2</sub> is utilized potentially as the primary electron acceptor. Once O<sub>2</sub> is depleted, anaerobic microorganisms typically use available electron acceptors in the following



**Table 2.** Summary of results of geochemical correlation parameters from tars and oils. Ratios are defined in the text.

Ratios are defined in the text.								
Site	Pr/Ph	m/z 191			m/z 217			RI
		$\frac{23T}{C_{30}}$	$\frac{Tm}{Ts}$	Triplet	$\frac{C_{30}}{C_{29}}$	$C_{29} \frac{S}{S+R}$	$\frac{C_{28}}{C_{29}}$	
Locally produced oils								
103	1.5	2.1	0.50	7.1	1.9	0.48	0.20	0.30
105	1.5	1.9	0.56	6.4	1.9	0.48	0.20	0.27
106	1.5	2.6	0.41	7.7	1.9	0.48	0.20	0.25
109	1.5	1.8	0.55	6.4	1.9	0.48	0.20	0.27
<u>Site A (02OS-)</u>								
AG020-22701	1.2	1.4	0.63	5.4	2.0	0.47	0.21	0.33
AG01-22702	1.2	1.3	0.63	5.6	1.9	0.46	0.20	0.40
AG020-22703	1.3	1.3	0.55	5.9	1.9	0.47	0.21	0.39
AG020-22801	nc*	1.2	0.57	5.8	1.9	0.47	0.20	0.46
AE12-436	1.3	1.4	0.62	5.7	1.9	0.47	0.20	0.62
AP01-61 cm, Sludge Pit	1.5	1.3	0.64	6.1	1.8	0.46	0.18	0.43
AP01-30.5 cm, Sludge Pit	1.5	1.3	0.60	6.0	1.9	0.45	0.20	0.40
AZ01	1.1	1.2	0.59	4.9	1.9	0.49	0.19	0.52
AHA04-20 cm	1.1	1.5	0.58	5.6	1.9	0.47	0.19	0.33
AHA04-46 cm	1.3	1.5	0.56	5.8	1.9	0.47	0.20	0.46
AHA04-66 cm	1.1	1.4	0.59	5.5	2.0	0.47	0.19	0.40
AGO20a-22802	1.4	0.70	0.63	3.6	1.7	0.58	0.20(?)	0.24
AGO20b-22802	1.6	0.23	1.2	3.1	1.8	0.43	0.33	0.74
AE03	nc*	0.56	0.52	1.2	1.8	0.61	0(no C <sub>28</sub> )	0.24
<u>Site B (02OS-)</u>								
BE07	1.4	1.1	0.66	5.0	1.8	0.47	0.20	0.47
BU-314	1.6	1.5	0.59	5.9	1.9	0.48	0.21	0.36
BU-314	1.5	1.4	0.56	5.5	1.9	0.47	0.21	0.39
BA03	1.4	1.2	0.62	4.8	2.0	0.48	0.22	0.78
BU-303	1.5	2.0	0.46	6.5	2.0	0.48	0.20	0.26
BU-317	1.4	2.2	0.48	6.8	1.9	0.47	0.20	0.62
312	1.3	1.0	0.76	3.1	1.8	0.46	0.28	0.78
313	1.4	1.1	0.75	4.8	2.0	0.47	0.20	0.68

\*nc = not calculatable due to lack of constituents; ? = uncertain identification of relevant peak(s)

order nitrate( $NO_3^-$ ), producing  $N_2$ , or ferric iron ( $Fe^{3+}$ ), producing ferrous iron ( $Fe^{2+}$ ), sulfate ( $SO_4^{2-}$ ), producing sulfide ( $S^{2-}$ ), and carbon dioxide ( $CO_2$ ), producing  $CH_4$ . By looking at both the spatial distribution of the different microbial populations present on the sediment and in the pore water; and the spatial distribution and concentration of oxidation/reduction reactants and products, the mechanism(s) and rates of biodegradation can be assessed. A reconnaissance for the different physiological groups of microorganisms, along with the ground-water geochemistry, can provide information on the major microbial processes that may be occurring in the subsurface environment. The microbial population can be categorized under the following physiological groups:

## **Aerobes**

Microorganisms that utilize  $O_2$  as electron acceptor are termed aerobes and those that can only use  $O_2$  as the electron acceptor are termed obligate aerobes. The use of  $O_2$  as the electron acceptor always provides the most energy from a given compound and microorganisms capable of using other electron acceptors will always use  $O_2$  first.

## **Facultative Aerobes**

Because diffusion of molecular oxygen in water is about 100,000 times slower than it is in the gas phase, an oxic system can quickly become anoxic. The next most thermodynamically efficient electron acceptor commonly found in the environment is nitrate ( $NO_3^-$ ). Nitrate, nitrite ( $NO_2^-$ ), and nitrous oxide ( $N_2O$ ) are used as electron acceptors by many common facultative aerobic soil bacteria. These microorganisms simply switch to using the next best electron acceptor ( $NO_3^-$ ) when  $O_2$  is not available. When  $NO_3^-$  has been utilized or is not present, most, but not all, of these microorganism can switch to  $Fe^{3+}$ , the next best electron acceptor.

## **Obligate Anaerobes (Respiratory)**

These microorganisms are the obligate iron reducers, sulfate reducers, and  $CO_2$ -reducing methanogens, each of which may be thought of as having a very truncated respiratory system. These organisms obtain very little energy from these processes, but still prevail under these meager conditions. The obligate iron and sulfate reducers can use acetate and/or molecular hydrogen ( $H_2$ ) as their energy source. The methanogenic microorganisms in this group use molecular  $H_2$  or formate as their energy source. The  $H_2$  is generated by the other group of anaerobes described immediately below.

## **Obligate Anaerobes (Fermentative)**

Fermentation, by definition, is the use of organic electron donor to give a stoichiometric balance of protons and electrons in lieu of an electron acceptor (*i.e.* oxidation and reduction of the same organic compound). Many of these microorganisms produce  $H_2$  during the production of protons and electrons from more complex native or WSF compounds. Acetate fermenting methanogens are also included in this group.

## **Site A Microbially Important Geochemistry**

Low molecular weight fatty acids (*e.g.*, butyrate, propionate, acetate and formate), known intermediates in the anaerobic microbial degradation of crude oil and other complex organic compounds (5), concentrations are very high (210 mg/L acetate, site AP-01) in the sludge pond near the road. These concentrations generally decrease moving downslope (4) suggesting anaerobic microbial processes predominate (AE-08, AE-09, and AE-10, generally 0.8-1.2 m below land surface [BLS]). The exception to this trend occurs in the deeper wells AA-03s (1.2-2.7 m BLS), and AA-03d (3.3-4.9 m BLS) where acetate concentrations reach 101 and 517 mg/L, respectively). Wells AA-04s (1.8-3.4 m) and AA-04d (5.8-7.3 m), located outside of the area affected by the sludge pits have non-detectable levels of fatty acids; however, they have high total dissolved solids (TDS) and been affected by produced water. The DOC concentrations generally positively correlate the concentrations of fatty acids. In addition to the volatile fatty acids,

low concentrations of benzene (0.29 mg/L) and toluene (0.34 mg/L) were found in a water sample from AP-01.

Sediment  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratios (14) outside the oil-impacted soils (from the old southern tank farm to north of the sludge pits, AE-04 to -07) at the site are low ( $< 0.5$ ) in shallow sediment (0-1.2 m, BLS) samples. The low  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratios result from low  $\text{Fe}^{2+}$  concentrations on the sediments suggesting a lack of crude oil components for microbial biodegradation and thus iron reduction; however, the  $\text{Fe}^{3+}$  concentrations at these sites range from 0.5 to 1.0 mg/g. The total iron concentration in the water sample from the sludge pit (AP-01) is 595 mg/L, strongly suggesting that microbial iron-reduction is occurring at the expense of the water-soluble components of the oil present in the pits.

In addition, very low levels of  $\text{NO}_3^-$  were found throughout the site (4), and no measurable amounts of  $\text{S}^{2-}$  were found even with the probable anoxic conditions as evidenced by the sulfate reducing microorganisms near the oil impacted soils and high concentrations of  $\text{SO}_4^{2-}$ . Only trace amounts of  $\text{CH}_4$  were detected in the water samples throughout the area and downslope near the sludge pits, again strongly suggesting that microbial iron reduction is the predominant microbial process in and around the sludge pit.

## Site A Microbial Populations

Microbial populations capable of aerobic growth are equal to or slightly larger than populations capable of fermentation suggesting that the population is mixed aerobic and facultative anaerobes (microorganisms capable of aerobic growth in the presence of  $\text{O}_2$ , and fermentation or either denitrification and/or iron-reduction in the absence of  $\text{O}_2$  (Table 3). This observation for the shallow samples AHA-01 to -04 is consistent with a flow system that is driven by rainfall events. The rainfall contains dissolved  $\text{O}_2$  and when the event has passed pore spaces with higher dissolved  $\text{O}_2$  will remain. Of the samples throughout this site, only a small proportion of the microorganisms are capable of denitrification (AE-10 and -12 did not have a denitrifying population); this would be due to very low levels of  $\text{NO}_3^-$  found at this site.

Presumably, in samples downslope from the sludge pit (AE-08, -10, and -12), anaerobic microorganisms should predominate. The iron reducing population at this site appears to be a mixture of facultative aerobes and strict anaerobic microorganisms with the strict anaerobic microorganisms predominating. This can be deduced from the difference in the numbers of iron reducing minus the numbers of denitrifiers. Relatively large numbers of microorganisms capable of sulfate reduction are present throughout the site; however, they may not be particularly active except in micro niches. Since concentrations of  $\text{S}^{2-}$  are very low even with the abundance of  $\text{SO}_4^{2-}$  at the site. Only very low numbers ( $< 100$  MPN/g) of methanogenic microorganisms were found at three sites (HA-01, HA-03 and AE-12) indicating they may not be particularly active except in micro niches near the sludge pits.

## Study Area Production Oils

The four oils from currently producing oil wells serve as representative baseline samples for oils produced throughout this local area. These four oils share similar characteristics. They are typical paraffinic-naphthenic light oils, with an API gravity of approximately 35, containing *n*-alkanes as their dominant components. They are fully mature oils with very similar chemical fingerprints, as shown by the correlation

**Table 3.** Sample locations and Most Probable Numbers (MPN) of microbial populations on sediment and in water samples.

Site #	Notes	Aerobes MPN/mL	Aerobes MPN/g	Fermentors MPN/mL	Fermentors MPN/g	Denitrifiers MPN/mL	Denitrifiers MPN/g
AE08A	0-1.2 m, bottom		3.5E+06		2.8E+05		8.8E+03
AE08B	0-1.2 m, 25 cm from top		>>		2.0E+05		8.7E+02
AE10	0-1.2 m, 25 cm from bottom		2.2E+05		1.1E+06		ND
AE12	0-1.2 m, 41 cm from top		1.4E+03		1.6E+05		ND
AHA01	~20-30 cm BLS		5.2E+05		1.7E+05		9.7E+01
AHA02	~20-30 cm BLS		1.8E+05		7.4E+04		ND
AHA03	~20-30 cm BLS		8.3E+05		2.5E+06		1.4E+02
AHA04	20 cm BLS		1.8E+05		2.8E+04		1.4E+02
AHA04	46 cm BLS		>>		8.7E+03		8.0E+01
AHA04	66 cm BLS		2.1E+06		1.5E+04		2.7E+01
BEW07	1.2 m BLS			7.9E+02		ND	
BE07A	0-1.2 m, 66.5 cm top	2.4E+03	9.9E+04		9.3E+02		ND
BE07B	1.2- 2.1 m, 35.5 cm top		4.6E+04		1.3E+04		3.8E+01
BE08	1.2-2.1 m, top		6.0E+03		4.4E+03		ND
BE09S	1.2-2.1 m, bottom Shale		2.6E+03		8.8E+02		ND
BE09	1.2-2.1, 26 cm top		2.4E+03		6.0E+03		ND
BE10	0-1.2 m, 70 cm top		8.4E+04		1.0E+03		ND
BE11	1.2-2.1 m, 40.5 cm top		5.7E+03		5.7E+03		ND
BE12	1.2-2.1 m, 15 cm top		7.2E+05		7.9E+05		ND

BLS = below land surface

ND = < 20-40/mL or g

>> = > E+6/mL or g

~~ = approximate value E+2-4/g

**Table 3. Cond't**

Site #	Notes	Iron		Sulfate		Sulfate Reducers		Total	
		Reducers MPN/mL	Reducers MPN/g	Reducers MPN/mL	Reducers MPN/g	MPN/mL	MPN/g	Methanogens MPN/mL	Methanogens MPN/g
AEO8A	0-1.2 m, bottom		2.0E+06			9.4E+05		1.0E+00	
AE08B	0-1.2 m, 25 cm from top		>>			5.0E+03		ND	
AE10	0-1.2 m, 25 cm from bottom		3.3E+06			2.6E+05		ND	
AE12	0-1.2 m, 41 cm from top		2.6E+05			2.6E+03		4.0E+00	
AHA01	~20-30 cm BLS		5.2E+05			1.8E+04		4.3E+00	
AHA02	~20-30 cm BLS		1.4E+05			7.4E+03		ND	
AHA03	~20-30 cm BLS		2.5E+06			1.4E+04		7.7E+01	
AHA04	20 cm BLS		9.2E+04			2.0E+03		ND	
AHA04	46 cm BLS		>>			1.5E+04		ND	
AHA04	66 cm BLS		>>			3.2E+05		ND	
BEW07	1.2 m BLS	2.4E+05		3.1E+02				7.0E+00	
BE07A	0-1.2 m, 66.5 cm top		1.3E+02			9.9E+02		ND	
BE07B	1.2-2.1 m, 35.5 cm top		4.6E+04			6.3E+03		2.5E+01	
BE08	1.2-2.1 m, top		ND			1.5E+02		ND	
BE09S	1.2-2.1 m, bottom Shale		ND			ND		ND	
BE09	1.2-2.1, 26 cm top		ND			1.4E+03		ND	
BE10	0-1.2 m, 70 cm top		4.9E+04			~		ND	
BE11	1.2-2.1 m, 40.5 cm top		1.4E+03			3.9E+02		ND	
BE12	1.2-2.1 m, 15 cm top		3.1E+06			1.8E+04		1.1E+02	

BLS = below land surface

ND = &lt; 20-40/mL or g

&gt;&gt; = &gt; E+6/mL or g

~~ = approximate value E+2-4/g

parameters given in Table 2. Small differences in these parameters allow some differentiation between the four oils, but their overall natures are quite similar. Even though petroleum production is from shallow sandstones (450-524 m-Site A and 260-305 m-Site B), these fresh oils show no sign of biodegradation, indicating that their reservoirs and the associated high salinity brines are not conducive to microbial action on the hydrocarbon phase.

However, as the oil has been mined, brought to the surface, and spilled throughout the study areas, the resultant oils exhibit widely varying levels of degradation. Volatilization, water washing, and microbial action are likely responsible for the transformation of the source oil to the surficial weathered, biodegraded, and sometimes asphaltic oils observed at the study sites.

## Characterization of Site A Oils

The majority of oil samples from Site A correlate closely with each other, despite being at highly variable stages of degradation (Tables 1 and 2). Three samples in a core at the top of the production area (AHA04, samples from 20 cm, 46 cm, and 66 cm BLS), most likely represent older oil spilled from earlier production. They are degraded to the point of complete loss of the *n*-alkanes ("C"-stage), with the sample lowest in the core showing the most degradation, containing a highly weathered component ("D") and only low levels of the isoprenoids characteristic of the C-stage. Degradation to the C-stage in a fuel oil spill is estimated to take at least 20 years (15).

Down slope, approximately 60-90 m from these samples, are eight samples from different areas of a sludge disposal pit. One area is obviously old and weathered, and its oils (22702, 22801) and oil floating on water from a well in its midsection (AE-12) are at the C- or D-stages. Another section, where the oil is fresher and probably more recently dumped, three oils (AP01 30 cm and AP01 60 cm, along with 22703) are at A-stage or slightly beyond, at an A/B-stage. Two samples from the downslope edge of the same pit area, where there is some overlap, are fairly fresh (22701, B/A-stage) and quite old (AZ01, D- with some C-stage), respectively. It is not known whether the eight pit-area oils migrated downslope from the production area over time or were dumped during and after production, but these eleven oils are clearly related (Table 2). However, the other three oils from this site are not so closely related. Samples a-22802 and b-22802, from two different horizons in a pit about 110 m further downslope, adjacent to the lake, are not the same as the upslope samples, and in fact, are somewhat dissimilar to each other. The first, from 10 cm depth, is a mix of highly degraded oil and fresh oil (D- and A-stages), and the second, from 40 cm depth, is quite fresh (A-stage). The old surface oil at the west edge of the site (AE03) is the most different, and probably is a remnant of a very old disposal pocket, possibly older than 100 years. Despite individual differences in the four different disposal pockets at Site A, all the oils reflect the characteristics of the local oil province in their biomarker distributions. In addition, however, there is perhaps some extraneous oil product at some sites. Samples from the core at the production site and from downslope in the old part of the sludge pit contain additional components, low levels of an anomalous group of high molecular weight *n*-alkanes. The two new sludge pit samples, AP01-30 cm and AP01-60 cm, also contain some high molecular weight *n*-alkanes, giving their GC/MS TIC two distribution maxima, the typical primary maximum at *n*-C<sub>18</sub> and another at *n*-C<sub>28</sub>. These sample sites, therefore, may also be contaminated with some extraneous oil product such as lubricating oil or the like.

There is no generally applied method to determine from the chemical profile whether the oil has been degraded aerobically or anaerobically. However, some recent work on crude oil spillage in Bemidji, Minn., suggests that in the latter stages of degradation, the profile of the homologous *n*-alkylcyclohexanes (*m/z* 83 chromatograms) can shed some light on this differentiation (17). These compounds are more refractory than *n*-alkanes, and thus retain their contribution to the chemical signature of the source oil for a longer period of time after exposure to the environment. Their original distribution in a fresh oil is usually bell-shaped (Figure 3B and 4). Weathering (aerobic degradation) attacks and removes the low molecular weight end of the series (16), whereas anaerobic biodegradation, particularly in the subsurface, slowly degrades homologs from the high molecular weight end (17). At Site A the samples show variable amounts of low-end loss, even in the relatively undegraded samples, indicating that all the samples have undergone some physical weathering or aerobic loss. However, the more degraded samples (at about the C-stage, but beyond this stage samples are too degraded to interpret) show some loss from the high molecular weight end as well, indicating some anaerobic losses also. This pattern occurs to some extent in the old fill-area (samples 22702 and AE12) and possibly in the upslope oil-impacted soil samples at AHA04.

## Site B Microbially Important Geochemistry

In contrast to Site A, the tank battery above the main pit is actively pumping oil and reinjecting the produced brine. A sample from the oil separation tank at this site (BU-314) contains indicators of microbial activity, in spite of the extremely high TDS (133,000 mg/L). In addition to acetate and formate (0.5 and 0.3 mg/L, respectively), benzene (4.0 mg/L), toluene (0.87 mg/L), ethylbenzene (0.73 mg/L), *m*- and *p*-xylene (0.71 mg/L), and *o*-xylene (0.43 mg/L) were detected in the water sample. The water sample also contained 70 mg/L ammonia, 35 mg/L total iron, and a lower pH (6.5) than other water samples in the area. These observations suggest the WSF of the oil is, even in the presence of extremely high TDS brines, undergoing biodegradation. The higher molecular weight fractions of the oil apparently do not biodegrade in the formation or in the oil separation system or tanks. These observations require further investigation before they can be confirmed.

Water samples from the main pit just in front of the tank battery (BU-317) also showed signs of microbial degradation of the WSF of the oil. In addition to high total dissolved iron (40 mg/L), higher total organic carbon (43 mg/L), and a lower pH (6.6), the sample contained acetate (0.6 mg/L) and formate (0.3 mg/L). Benzene (0.23 mg/L) was also detected. Down slope away from the main pit to the lake, only low concentrations of acetate and formate ( $\leq 0.6$  mg/L) were found.

Sediment  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratios (13) at this site are high ( $\sim 45$  to  $\sim 11$ ) in shallow (0-1.2 m) samples near the lake water line and below the main pit (BE-07, BE-09, BE-11, and BE-16) strongly suggesting microbial iron reduction. The  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratios in deeper samples (1.2-2.1 m) in the same holes and all samples further from the shore and the main pit have ratios generally  $< 1$ , with the exception of BE-09 (12.6). This well is just downslope from the main pit and appears to be in the flow path from the main pit to the lake. The low values for wells closer to the lake and out of the flow path suggests that these sites are influenced by the  $\text{O}_2$ -rich lake water and aerobic microbial processes may predominate.

As at Site A, very low levels of  $\text{NO}_3^-$  were found throughout the site (4), no measurable amounts of  $\text{S}^{2-}$  were found, even with the probable anoxic conditions as evidenced by the sulfate reducing microorganisms near the oil impacted soils and high concentrations of  $\text{SO}_4^{2-}$ . Only trace amounts of  $\text{CH}_4$  were detected in the water samples throughout the area and downslope near main pit, again strongly suggesting that microbial iron reduction is the predominant microbial process in and around the main pit to the lake.

## **Site B Microbial Populations**

Microbial populations capable of aerobic growth are equal to or less than populations capable of fermentation suggesting that the population is predominantly facultative aerobes (Table 3). Only one sample, BE-07 at 1.2 to 2.1 m, at this site contained microorganisms (38 MPN/g) capable of denitrification, again due to the very low levels of  $\text{NO}_3^-$  found at this site. The iron reducing population would be strictly anaerobic since there are not a significant numbers of denitrifiers at this site.

Relatively large numbers of microorganisms capable of sulfate reduction are present throughout; however, they may not be particularly active. This observation is suggested by the very low concentrations of  $\text{S}^{2-}$  even with the abundance of  $\text{SO}_4^{2-}$  at the site.

Very small numbers ( $< 100$  MPN/g) of methanogenic microorganisms were found at only two sites (BE-07 and BE-12) indicating they may not be particularly active, as is the case with the sulfate reducing microorganisms. The water sample from BE-07 has a population that is essentially the same as the two sediment samples from this site. The fact that the total population is evenly distributed between both the water and the sediment strongly suggests that the population is quite active. In situations where little or no microbial activity is taking place, up to 90% of the population is attached to the sediment (1). In contrast to this finding, the sample taken in the shale (BE-09S) has a population composed of only aerobic and fermenting microorganisms. Denitrifying, iron reducing, sulfate reducing, and methanogenic microorganisms were not detected in this sample. This may be due to the overall impermeability of the shale and will be investigated in greater detail.

## **Characterization of Site B Oils**

Samples from Site B show more variation in their correlation parameters, indicating that the oils are probably from several slightly different collection sources. Two samples of fresh oil (BU-314, congealed oils from the brine tank battery, at degradation state A) correlate with each other, and perhaps are similar to those in the main body of oils from Site A (Table 2). Two other fresh oil samples from the main pit just in front of the tank battery (sample BU-317 and -303), which might be expected to be the same as the oils from the brine tanks, actually have slightly different parameters although they are at the same A-state of degradation. A C-degraded oil, BE07, from a subsurface core of wet soil, about 50 m in front of the main pit (sometimes submerged under the lake water), is again similar but not identical to that from the brine tanks or the main pit. Another sample from the subsurface, BA03, present in low amounts on rocks at almost 5.8 m below land surface, is also at the C-stage and is similar to the oil in BE07 except for one parameter. The two oils associated with flocculent material from the deep



well just off-site west (312 and 313) are C-degraded and correlate fairly well with one another, and, again, are slightly different from the other oils.

With respect to the type of degradation that has impacted the samples, all four A-state oils (from the brine tanks and the main pit) show only low-end, aerobic or weathering loss on the *n*-alkylcyclohexane chromatograms. The other four samples, all at C-degradation, show both low-end and high-end losses, indicating both weathering and anaerobic biodegradation. Examples are shown in Figure 4.

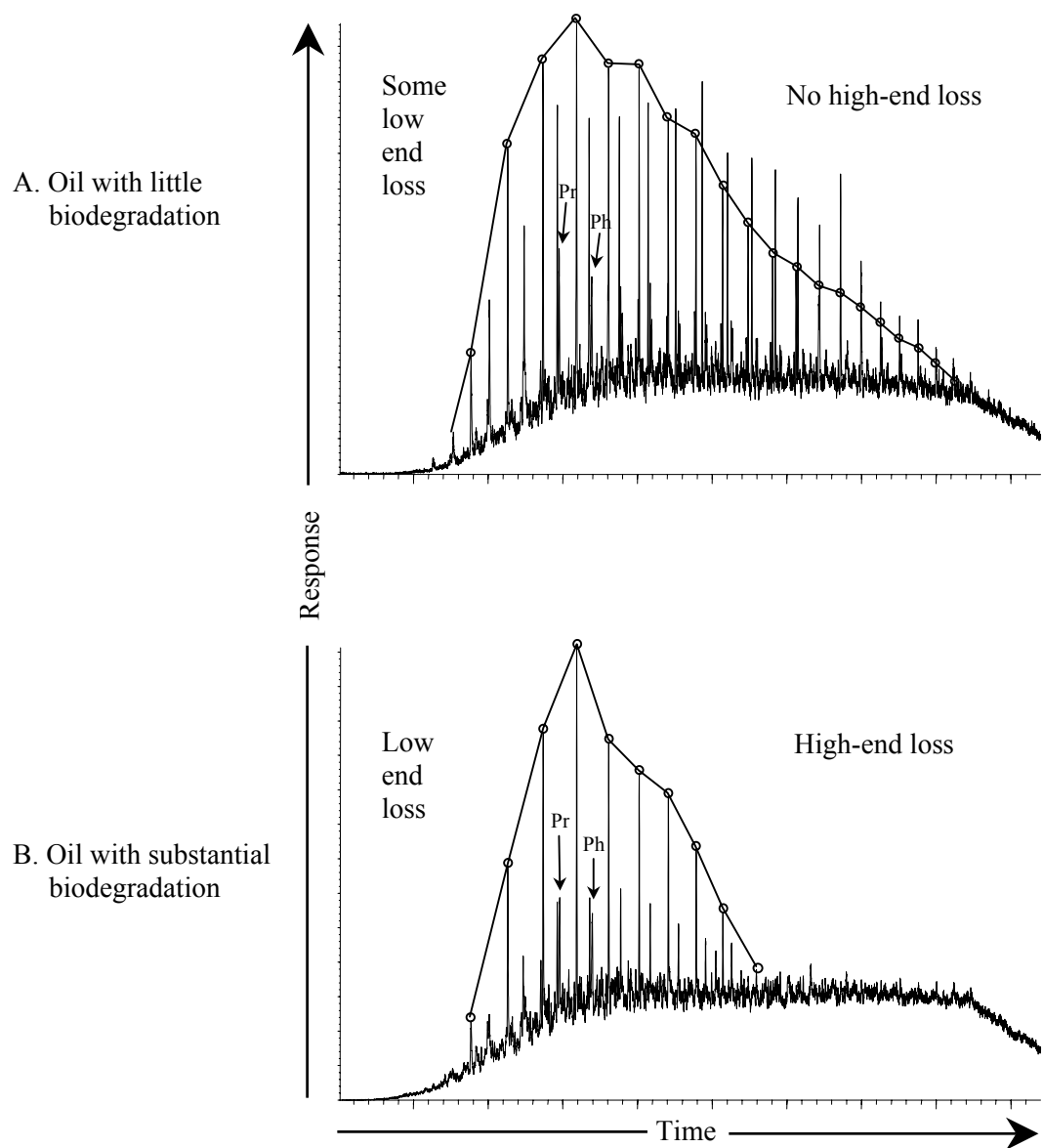
## CONCLUSIONS

At both sites, the microbial populations are degrading the low molecular weight WSF crude oil compounds. This fractions includes low molecular weight alkanes, one and two ring aromatic compounds, phenolic compounds, BTEX compounds, and *n*-alkylcyclohexanes. These fractions along with some anthropogenic carbon are being used by the resident microbial populations as electron donors, extracting the energy available, and reducing the appropriate inorganic electron acceptors for that population (with the exception of the fermenting populations). Both the geochemical evidence and make-up of the microbial populations in the oil-impacted soils at Sites A and B suggests that the systems are thermodynamically poised at the level of iron reduction.

Because the shallow system upslope from the sludge pit at Site A is apparently rainfall driven (intermittent rainfall dissolved O<sub>2</sub> input), the microbial populations, in general, are comprised of aerobes, facultative aerobes, and strict anaerobes. However, the high concentrations of volatile fatty acids and high dissolved iron and other geochemical parameters of the system near and just downslope of the sludge pit suggests that the microbial population should be predominantly facultative aerobes and strict anaerobes, with iron reducing microorganisms predominating. This was confirmed by microbial population analyses. In addition, the population of sulfate reducers is only an order of magnitude less the iron reducers and the potential for a more active sulfate reducing population cannot be ruled out.

In contrast to Site A, it appears that the microbial populations are exclusively comprised of facultative aerobes and strict anaerobes at Site B, due to the water-saturated soils throughout the site. The hydraulic pressure from the main pond and the lake would tend to keep these soils saturated. It is unclear at this time the effect of the fluctuating water levels and input of anthropogenic carbon from the lake has on the nature of the microbial populations and the overall degradation pathways at this site.

Oil residues throughout the study areas are all similar in their general characteristics, indicating that they are all from the same regional oil province. There are, however, enough small differences in correlation parameters within the oil samples that several slightly different sub-groups of oils can be observed. Thus, it appears that at Site A all the oils in the roughly 100 m area from the oil-impacted soil where original production occurred, downslope to where the oil either flowed or was dumped, is the same oil, although at significantly variable stages of biodegradation. Oil samples from the three other, more distant sites are somewhat different and thus probably represent



**Figure 4.** m/z 83 (*n*-cyclohexane) profile showing an A-degradation sample (BU-317) and a C-degradation sample (BE-07). Also, compare Figure 3A with Figure 3B at a similar degradation level.

o o o o o o o *n*-alkylcyclohexane peaks

different oil spillages. Oils that give possible evidence of anaerobic degradation are the four oils at the C-degradation level. These occur generally beneath land surface (the core at the upslope production site and the well sample in the fill area), except for 22702, which is from a surface mud in the older part of the fill area. In samples in a D-degradation, the oils are too degraded for the method outlined in this work to determine the nature of the degradation.

At Site B the samples sort out in small groupings-two from the brine tanks, two from the main pit, and two from a deep well, then one downslope from the pit and one NW of the pit, the last two from the subsurface. There does not seem to be a correlation between the oils at closely adjacent sites, that is, oil in the brine tanks, the main pit, or the downslope sample, indicating that there probably is not significant subsurface transport of the non-water soluble, or low molecular weight components of the spilled oils. As at Site A, the samples which show tentative evidence of anaerobic degradation are the subsurface oils at the C-degradation stage, that is, the last four listed above.

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